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Common Subtypes of Idiopathic Generalized Epilepsies: Lack of Linkage to D20S19 Close to Candidate Loci (EBN1, EEGV1) on Chromosome 20

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Hereditary factors play a major role in the etiology of idiopathic generalized epilepsies (IGEs). A trait locus (EBN1) for a rare subtype of IGEs, the benign neonatal familial convulsions, and a susceptibility gene (EEGV1) for the common human low-voltage electroencephalogram have been mapped close together with D20S19 to the chromosomal region 20q13.2. Both loci are potential candidates for the susceptibility to IGE spectra with age-related onset beyond the neonatal period. The present study tested the hypothesis that a putative susceptibility locus linked to D20S19 predisposes to spectra of IGEs with age-related onset from childhood to adolescence. Linkage analyses were conducted in 60 families ascertained through IGE patients with juvenile myoclonic epilepsy, juvenile absence epilepsy or childhood absence epilepsy. Our results provide evidence against linkage of a putative susceptibility gene for four hierarchically broadened IGE spectra with D20S19 assuming tentative single-locus genetic models. The extent of an "exclusion region" (lod scores below − 2) varied from 0.5 cM up to 22 cM on either side of D20S19 depending on the trait assumed. These results are contrary to the expectation that a susceptibility gene in vicinity to D20S19 confers a common major gene effect to the expression of IGE spectra with age-related onset from childhood to adolescence. © 1996 Wiley-Liss, Inc.

KEY WORDS: idiopathic generalized epilepsies, genetics, linkage, candidate genes, chromosome 20

INTRODUCTION

Family and twin studies demonstrate the major role of genetic factors in the etiology of idiopathic generalized epilepsies (IGEs), which have a prevalence of 0.6% in the general population and represent 40% of all epilepsies [Gedda and Tatarelli, 1971; Blandfort et al., 1987; Berkovic et al., 1994; Beck-Mannagetta and Janz, 1991]. Seven distinct IGE subtypes have been delineated by clinical and eletroencephalographical (EEG) characteristics of their seizure types and their age-related onset [Commission on Classification and Terminology of the International League Against Epilepsy, 1989]. Juvenile myoclonic epilepsy (JME), juvenile absence epilepsy (JAE) and childhood absence epilepsy (CAE) represent common IGE subtypes with an age-dependent onset between childhood and adolescence [Janz, 1989; Wolf, 1992a,b; Loiseau, 1992]. This group of IGE syndromes is suitable for linkage studies because of their distinct seizure types, the frequent familial clustering and the evidence for a shared genetic predisposition [Berkovic et al., 1987, 1994; Beck-Mannagetta and Janz, 1991; Janz et al., 1992]. Furthermore, generalized epileptogenic EEG discharges are present in up to 30% of otherwise healthy family members, making this a potential subclinical trait marker [Metrakos and Metrakos, 1961; Doose et al., 1973; Tsuboi and Endo, 1977; Noebels, 1991; Pedley, 1991].

Despite clear familial aggregation of the common IGEs with age-related onset from childhood to adolescence, their mode of transmission is yet unknown, even when generalized epileptogenic EEG abnormalities are considered as a subclinical trait marker. Several lines of evidence suggest that multiple genes are involved in the inheritance of the common IGEs [Greenberg et al.,

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1992; Delgado-Escueta et al., 1994]. One susceptibility gene (EJM1) predisposing to a broad IGE spectrum has been assigned to the short arm of chromosome 6 in families ascertained through JME patients [Greenberg et al., 1988; Greenberg and Delgado-Escueta, 1993; Durner et al., 1991; Weissbecker et al., 1991]. However, evidence for genetic heterogeneity among families of JME patients [Whitehouse et al., 1993; Delgado-Escueta et al., 1994] and lack of a major gene effect of EJM1 in families ascertained through patients with CAE or JAE [Serratosa et al., 1993; Sander et al., 1995] implicate that other loci, besides that identified on chromosome 6, confer susceptibility to the common IGEs with age-related onset from childhood to adolescence.

A critical first step towards the elucidation of the complex inheritance of the common IGEs is the detection of trait loci of monogenic epilepsy syndromes and the identification of genes involved in the regulation of neuronal activity in the human brain [Lindhout et al., 1992; Delgado-Escueta et al., 1994]. Two trait loci responsible for the *rare benign* neonatal familial convulsions (EBN1) and the common human low voltage EEG (EEGV1) represent such high-ranking candidate genes. EBN1 and EEGV1 are tightly linked to the anonymous DNA marker D20S19 on the chromosomal segment 20q13.2 [Leppert et al., 1989; Malafosse et al., 1992; Ronen et al., 1993; Steinlein et al., 1992]. At present, it remains an open question whether EBN1 and EEGV1 are two distinct trait loci or whether both phenotypes are caused by different mutations of the same gene. The human low-voltage EEG is a normal EEG variant with an autosomal dominant mode of inheritance [Vogel, 1962]. It is characterized by the absence of an alpha rhythm from the resting EEG. The benign neonatal familial convulsions (BNFC) represent a dominantly inherited subtype of IGEs. Clinical features are clonic or apneic seizures manifesting mostly on the second and third day of life and a favorable prognosis [Plouin, 1992]. The majority of BNFC families show linkage to EBN1 on chromosome 20q. However, evidence for clinical and genetic heterogeneity and linkage to markers on chromosome 8q has been demonstrated in two BNFC families [Lewis et al., 1993; Steinlein et al., 1995]. Approximately 16% of BNFC patients exhibit seizures beyond the neonatal period [Ryan et al., 1991; Ronen et al., 1993]. This finding suggests that EBN1 or genes in the vicinity of D20S19 might be involved in the genetic predisposition of broader spectra of IGE syndromes.

Our present linkage study tested the hypothesis that a D20S19-linked susceptibility locus contributes to the expression of broader IGE spectra in 60 families ascertained through IGE patients with JME, JAE or CAE.

MATERIALS AND METHODS

Family Ascertainment

The collection of families was performed as a collaborative effort of the Department of Neurology at the Free University of Berlin/Germany (n = 49), the Genetic Collaborative Group of the Italian League Against Epilepsy (n = 5) and the Department of Neurology at the University Innsbruck/Austria (n = 6). All 60 families were ascertained through patients with JME, JAE or CAE. Families were preferentially selected where at least one first-degree relative had idiopathic generalized seizures or had generalized spike-wave discharges in the resting EEG (GSW-EEG).

Clinical Characterization

Sixty families with 564 individuals were included in our analysis (Fig. 1). Genotypic data were obtained for 453 individuals. Clinical and EEG data for each individual were documented in a standardized anonymous protocol that was reviewed by experienced epileptologists (D. J., G. B-M.). The diagnoses of epilepsies and epileptic syndromes were performed according to the revised "Classification of Epilepsies and Epileptic Syndromes of the International League Against Epilepsy" [Commission on Classification and Terminology of the International League Against Epilepsy, 1989]. EEG data were available for 433 family members. Twenty-one out of 283 (7.4%) clinically healthy family members exhibited GSW discharges in their resting EEG.

Subgrouping of Families

Families were subdivided to provide additional homogeneity. Three partially overlapping subgroups of families were formed, comprising families with a family member affected by JME (n = 29), JAE (n = 28) or CAE (n = 20). This procedure restricts the phenotypic spectrum within each subgroup by including an obligate IGE phenotype in the family selection criteria, but also reflects the individual and familial phenotypic overlap of the IGEs with age-related onset from childhood to adolescence.

DNA Analyses

Samples of 7 μg genomic DNA, prepared from venous blood lymphocytes or lymphoblastoid cell lines were digested to completion with TaqI using five units of enzyme per μg of DNA. Restriction fragment length polymorphisms (RFLPs) were detected by Southern analysis using standard techniques [Sambrook et al., 1989]. The VNTR (variable number of tandem repeats)-probe pCMM6 (D20S19) [Nakamura et al., 1988] was labelled to high specific activity using the random-primer method [Feinberg and Vogelstein, 1983]. Genotyping was performed blind to the affection status. Paternity of the family members was confirmed by multilocus genotyping.

Classification of the Affection Status

Four hierarchically broadened IGE spectra were defined because of the unknown phenotypic variance of the putative trait locus. These four diagnostic schemes were delineated from family studies investigating the syndrome-related genetics of IGE phenotypes [Beck-Mannagetta and Janz, 1991; Janz et al., 1992; Italian League Against Epilepsy Genetic Collaborative Group, 1993] and from models applied in linkage analyses of families ascertained through JME patients [Durner et al., 1991; Weissbecker et al., 1991]. Individuals with the following phenotypes were classified as "affected" under four hierarchically broadened IGE spectra:

Model 1: JME, or JAE or CAE; (n = 103 affected individuals).
Model 2: IGEs, including JME, JAE, CAE, idiopathic epilepsies with generalized tonic clonic seizures (GTCS) on awakening (EGMA), idiopathic epilepsies with GTCS (EGTCS) and single idiopathic GTCS exhibiting interictally a GSW-EEG; (n = 129 affected individuals).
Model 3: IGEs or at least one idiopathic GTCS; (n = 138 affected individuals).
Model 4: IGEs, or at least one idiopathic GTCS or GSW-EEG; (n = 159 affected individuals).

Under each model, healthy family members or individuals with other kinds of epilepsies or epileptic seizures or EEG abnormalities, who were not considered to be "affected" under any of the models, were classified as "unaffected." Individuals with broader spectrum diagnoses were classified as "unknown" under more stringent models. Family members with insufficient clinical data were classified as "unknown" throughout. Family members for whom EEG data were lacking were classified as "unknown" under model 4. Affection status was classified independently by two experienced epileptologists (D. J., T. S.) without knowledge of the genotypic results.

Linkage Analysis

Linkage analyses were performed using the programs MLINK and ILINK from the LINKAGE package (V 5.1) [Lathrop and Lalouel, 1984]. The analyses were carried out under single-locus approximation models assuming either an autosomal dominant or a recessive mode of inheritance (MOI) with penetrance values of 30% and 50% for model 1, 50% and 70% for models 2 and 3, and 70% and 90% for model 4 [Vieland et al., 1992; Schork et al., 1993]. Eight liability classes were defined to allow modeling for an age-at-onset correction. Thereby, empirically determined age-dependent penetrances weighted the diagnostic certainty of unaffected family members who were potentially too young to exhibit the diagnostic criteria. A phenocopy rate of 0.01 was used throughout. Lod scores were calculated for equal recombination fractions in both sexes. With regard to computational limitations, eight alleles of D20S19 were taken into account with uniform allele frequencies of 0.125. Linkage analyses were carried out for all families and for three more homogeneous subsets of families selected through family members with JME (n = 29), JAE (n = 28) or CAE (n = 20). Frequencies of the susceptibility allele were estimated from the trait prevalence (0.3% for JME, JAE, and CAE; 0.6% for IGEs; 3% for IGEs or GSW-EEG), correcting for the penetrance values used. The homogeneity of the linkage data was tested by the admixture test (A-test) of the program HOMOG [Ott, 1983].

RESULTS

Fifty-seven out of 60 IGE families were at least partially informative for the VNTR polymorphism D20S19, which revealed 15 different alleles with band sizes between 1.1 and 10 kb. The two-point lod scores (Z) for linkage analyses between D20S19 and a putative IGE susceptibility locus are summarized in Table I. Varying penetrance values in the assumed range did not result in significant changes of the operational "exclusion regions" (lod score below − 2) for the selected trait models. To assure that the assumption of uniform marker allele frequencies did not provide false-positive evidence for linkage in the presence of untyped individuals [Ott, 1992], linkage analyses were additionally carried out assuming marker allele frequencies deduced from the genotype scores of 56 healthy married-in family members (data not shown). The comparison of the corresponding lod scores did not indicate a substantial influence of the marker allele frequencies in our data set.

For the entire group of families, the extent of an operational "exclusion region" varied from 0.5 cM up to 22 cM on either side of D20S19, depending on the trait model under study. Slightly positive lod scores were observed at recombination fractions (θ) above 0.10 under models 1 and 2 assuming an autosomal recessive mode of inheritance with reduced penetrance. A maximum lod score of $Z_{max} = 0.70$ was obtained at $\theta_{max} = 0.21$ under model 1 assuming an autosomal recessive MOI with 50% penetrance.

For each of the three partially overlapping subsets of families selected through patients with, respectively, JME, JAE or CAE, consistently negative lod scores were obtained at the D20S19 locus under each of the genetic models assumed. Lod scores below − 2 were reached throughout at the marker locus under trait models 2, 3 and 4. Slightly positive lod scores were observed at θ greater than 0.15 for each of the three family subsets under models 1 and 2 assuming an autosomal recessive MOI with reduced penetrance. Considering the expected information content of each family subset under the assumed trait models, the relatively highest lod score of $Z_{max} = 0.57$ was obtained at $\theta_{max} = 0.15$ for families ascertained through CAE patients, when family members with JME, JAE or CAE were considered as affected (model 1).

The A-test provided no evidence for heterogeneity among the entire group of families or any of the three subsets of families.

DISCUSSION

An appropriate IGE phenotype [Delgado-Escueta et al., 1991] and an efficient sampling strategy [Goldin et al., 1991] are essential requirements for the detection of a single susceptibility gene within the common IGE syndromes. JME, JAE and CAE exhibit overlapping complex phenotypes with an age-dependent onset, suggesting that the predisposition to this phenotypic spectrum is complex, that shared genetic factors contribute to the phenotypic variance, and that some of the genes determining phenotype are very common [Berkovic et al., 1987, 1994; Janz et al., 1992]. From a neurobiological point of view, these IGE subtypes appear to form a biological continuum with an age-dependent phenotypic expression due to a predominantly genetically determined impairment of postnatal brain maturation [Shinnar and Moshe, 1991; Wolf,

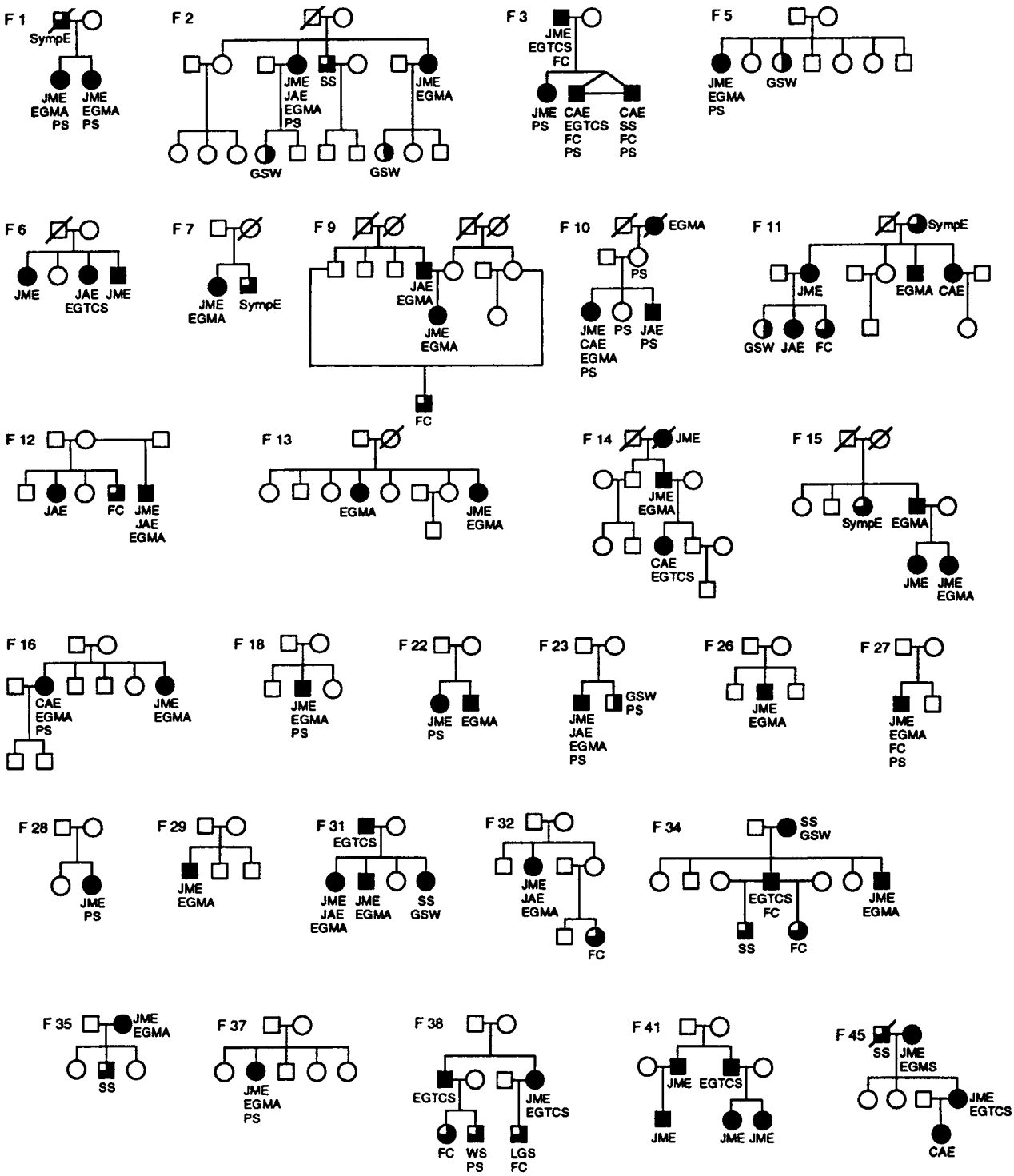
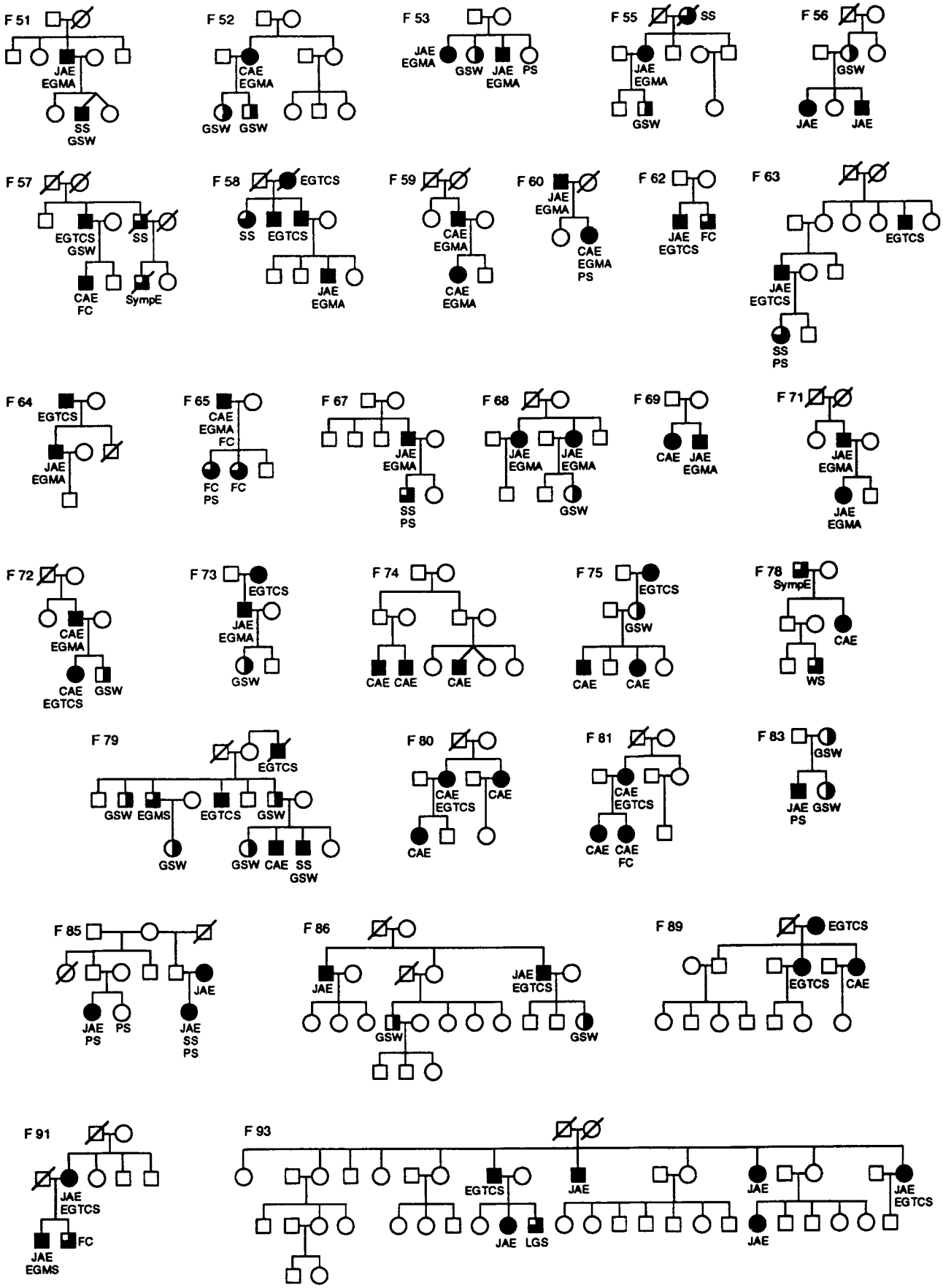


Fig. 1. Pedigrees of the 60 families ascertained through patients with JME, JAE or CAE. □ ○ = clinically unaffected individual; male, female; ■ ● = affected with an IGE; ◪ ◕ = affected with a non-IGE epilepsy or seizure; ◨ ◑ = asymptomatic individual with GSW-EEG; ⍁ ⌀ = deceased individual. Abbreviations: JME, juvenile myoclonic epilepsy; JAE, juvenile absence epilepsy; CAE, childhood absence epilepsy; EGMA, idiopathic epilepsy with GTCS on awakening; EGTCS, idiopathic generalized epilepsy with GTCS; GTCS, generalized tonic clonic seizure; EGMS, epilepsy with GTCS during sleep; LGS, Lennox-Gastaut syndrome; WS, West syndrome; SympE, symptomatic epilepsy or seizure; SS, single idiopathic GTCS; FC, febrile convulsion; GSW, generalized spike and wave discharges in the EEG; PS, photosensitivity in the EEG.


F 51
JAE
EGMA
SS
GSW
F 52
CAE
EGMA
GSW GSW
F 53
JAE
EGMA
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JAE
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PS
F 55
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JAE
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EGTCS
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F 91
JAE
EGTCS
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F 93
EGTCS
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JAE
EGTCS
JAE

Fig. 1

TABLE I. Two-Point Lod Scores of Linkage Analyses Between a Putative Susceptibility Locus for Four Hierarchically Broadened IGE Spectra (M1–M4) and D20S19 Assuming Either an Autosomal Dominant (AD) or Recessive (AR) Mode of Inheritance (MOI) With Reduced Penetrance (P%) for Various Recombination Fractions (θ)

		IGE fam. (n = 57)						
		$\theta_m = \theta_f$						
Model/MOI/P%		0.00	0.01	0.05	0.10	0.15	0.25	0.35
M1[a]	AD 30	−2.28	−1.85	−0.95	−0.41	−0.11	0.11	0.11
	AD 50	−2.93	−2.38	−1.27	−0.59	−0.22	0.09	0.11
	AR 30	−3.47	−2.75	−0.99	0.05	0.49	0.59	0.30
	AR 50	−4.38	−3.41	−1.19	0.03	0.54	0.65	0.33
M2[b]	AD 50	−7.02	−5.95	−3.64	−2.16	−1.28	−0.37	−0.04
	AD 70	−7.61	−6.45	−3.95	−2.33	−1.37	−0.37	−0.03
	AR 50	−6.20	−5.10	−2.47	−0.86	−0.07	0.39	0.25
	AR 70	−7.25	−5.79	−2.58	−0.79	0.06	0.51	0.31
M2[c]	AD 50	−9.18	−7.81	−4.78	−2.84	−1.70	−0.55	−0.14
	AD 70	−10.54	−8.94	−5.51	−3.29	−1.97	−0.61	−0.13
	AR 50	−8.99	−7.72	−4.43	−2.21	−1.00	−0.02	0.11
	AR 70	−11.98	−9.91	−5.35	−2.56	−1.10	−0.04	0.16
M4[d]	AD 70	−13.63	−11.37	−6.99	−4.14	−2.43	−0.68	−0.07
	AD 90	−21.53	−17.47	−11.13	−6.96	−4.38	−1.56	−0.39
	AR 70	−12.01	−10.32	−6.06	−3.19	−1.57	−0.19	0.07
	AR 90	−18.53	−15.05	−8.27	−4.20	−2.01	−0.20	0.11
		JME fam. (n = 29)						
		$\theta_m = \theta_f$						
Model/MOI/P%		0.00	0.01	0.05	0.10	0.15	0.25	0.35
M1	AD 50	−2.69	−2.17	−1.15	−0.56	−0.24	0.04	0.08
	AD 50	−3.16	−2.55	−1.15	−0.36	0.01	0.21	0.12
M2	AD 70	−5.53	−4.59	−2.67	−1.49	−0.81	−0.15	0.05
	AR 70	−4.06	−3.24	−1.53	−0.58	−0.11	0.19	0.13
M3	AD 70	−7.21	−6.18	−3.91	−2.40	−1.48	−0.50	−0.11
	AR 70	−7.50	−6.28	−3.70	−2.09	−1.17	−0.30	−0.04
M4	AD 90	−10.14	−8.95	−5.81	−3.61	−2.21	−0.67	−0.06
	AR 90	−9.77	−8.35	−5.20	−3.06	−1.77	−0.52	−0.11
		JAE fam. (n = 28)						
		$\theta_m = \theta_f$						
Model/MOI/P%		0.00	0.01	0.05	0.10	0.15	0.25	0.35
M1	AD 50	−1.16	−1.05	−0.70	−0.41	−0.24	−0.07	−0.02
	AR 50	−2.99	−2.38	−0.93	−0.11	0.26	0.38	0.21
M2	AD 70	−4.67	−4.13	−2.84	−1.90	−1.28	−0.55	−0.19
	AR 70	−3.09	−2.35	−0.73	0.12	0.47	0.52	0.26
M3	AD 70	−6.95	−6.19	−4.31	−2.89	−1.95	−0.82	−0.27
	AR 70	−4.51	−3.62	−1.64	−0.50	0.04	0.34	0.20
M4	AD 90	−11.59	−10.28	−7.05	−4.64	−3.03	−1.13	−0.24
	AR 90	−9.67	−8.14	−4.81	−2.61	−1.36	−0.23	0.03
		CAE fam. (n = 20)						
		$\theta_m = \theta_f$						
Model/MOI/P%		0.00	0.01	0.05	0.10	0.15	0.25	0.35
M1	AD 50	−0.31	−0.09	0.28	0.43	0.46	0.37	0.20
	AR 50	−0.96	−0.80	0.14	0.48	0.57	0.44	0.20
M2	AD 70	−2.38	−1.83	−0.71	−0.11	0.17	0.31	0.21
	AR 70	−2.16	−1.61	−0.41	0.20	0.43	0.42	0.21
M3	AD 70	−3.70	−2.77	−1.13	−0.34	0.02	0.20	0.12
	AR 70	−3.27	−2.66	−1.20	−0.32	0.09	0.28	0.15
M4	AD 90	−7.77	−5.44	−3.16	−1.85	−1.20	−0.54	−0.27
	AR 90	−4.75	−3.35	−1.07	0.01	0.44	0.53	0.27

**The linkage results are given separately for all 57 informative families and for three partially overlapping subgroups of families ascertained through cases with juvenile myoclonic epilepsy (n = 29), juvenile absence epilepsy (n = 28), and childhood absence epilepsy (n = 20).

[a]M1: JME, JAE or CAE.

[b]M2: IGEs.

[c]M3: IGEs, or idiopathic GTCS.

[d]M4: IGEs or idiopathic GTCS or GSW-EEG.

1992a]. Therefore, monogenic trait loci causing an impaired brain maturation, such as EBN1 and EEGV1, represent high-ranking candidate genes.

Our two-point linkage analyses in 57 informative families provide no evidence for an IGE susceptibility locus in the chromosomal region 20q13.2. The extent of an operational "exclusion region" (lod score below − 2) varied from 0.5 cM up to 22 cM on either side of D20S19, depending on the trait model under study. Slightly positive lod scores were observed at θ above 0.10 under models 1 and 2 assuming an autosomal recessive mode of inheritance with reduced penetrance. A maximum lod score of $Z_{max} = 0.70$ at $\theta_{max} = 0.21$ was found under an autosomal recessive MOI with 50% penetrance when individuals with JME, JAE or CAE were considered as affected. The A-test provided no evidence for heterogeneity among the families. However, the sensitivity of the A-test could be insufficient to reveal statistically heterogeneity due to the small information content of the included families. The presence of genetic heterogeneity could lead to a false exclusion of an existing linkage. To provide additional homogeneity, we performed linkage analyses separately for three subsets of families selected through patients with respectively, JME, JAE or CAE. This subgrouping procedure narrows the familial genetic background by including an obligate IGE phenotype, but also allows for a partial overlap among the family subsets due to the familial clustering and individual combination of JME, JAE and CAE (Fig. 1).

For each of the three family subsets, consistently negative lod scores were obtained at the D20S19 locus under each trait model studied. An operational "exclusion" was reached under diagnostic schemes 2, 3 and 4, regardless of the assumed genetic model. Families of JME patients showing no linkage to TaqI-RFLPs at the HLA-DQ locus (EJM1) did not show linkage to D20S19 (data not shown). A tentative hint for linkage was found under an autosomal recessive MOI with reduced penetrance in the three family subsets. Families of CAE patients provided more positive lod scores compared to the information content expected from families of JAE or JME patients. For families of CAE patients, a maximum lod score of 0.57 was obtained at $\theta_{max} = 0.15$ when family members with JME, JAE or CAE were included into the affection status. The A-test revealed no evidence for heterogeneity among the family subsets. Keeping in mind that we have performed multiple testing, the observed slightly positive lod scores could be a spurious hint of linkage. However, it might be worthwhile to perform further multipoint analyses with highly informative DNA markers from a chromosomal region 10 to 20 cM on either side of D20S19. An interesting candidate gene within this chromosomal region represents the gene encoding the human neuronal nicotinic acetylcholine receptor α4 subunit (CHRNA4) which has been mapped to chromosome 20q13.2-q13.3 [Steinlein et al., 1994]. The detection of a nonsence mutation within the α4 subunit gene cosegregating with BNFC in a single family revealed the first example of an IGE caused by a defective neurotransmitter receptor [Beck et al., 1994]. Linkage studies of families ascertained through CAE patients could be most suitable for testing the hypothesis whether our results indicate a potential IGE susceptibility locus with a possible age-dependent gene effect contributing to the expression of childhood epilepsies.

In contrast to the present exclusion results, D20S19 was tightly linked to EBN1 and EEGV1 ($\theta_{max} = 0.00$). However, the methodological problems in the parametric linkage analysis of genetically complex traits limit the strength of exclusion results. Diagnostic misclassification, genetic heterogeneity, phenocopies, incomplete and age-dependent penetrances, wrong genetic models or the lack of a sufficiently frequent major gene effect could lead to a false exclusion of an existing susceptibility gene. Alternatively or in addition to parametric analyses, nonparametric methods, like the affected sib-pair method, can be a powerful tool in linkage analysis of genetically complex traits where little reliable information about the mode of inheritance is available [Twellinger and Ott, 1994]. However, the nonparametric analyses are often not feasible because of the lower statistical power. We have tried to avoid modeling errors of the underlying mode of inheritance by conducting multiple tests with a broad range of single-locus approximation models, incorporating a phenocopy rate and an age-at-onset correction. By modeling an empirically deduced age-dependent penetrance of each diagnostic scheme for family members who were potentially too young to exhibit the diagnostic criteria and by classifying broader spectrum diagnoses as unknown under more stringent diagnostic schemes, we have weighted the diagnostic certainty of unaffected individuals. Even in the case of multiple testing which increases the chance of type 1 errors, we found no evidence of linkage to the D20S19 locus under all four diagnostic methods and a broad range of genetic models. These findings strengthen the validity of our negative results and justify the conclusion that a susceptibility gene in vicinity to D20S19 in the chromosomal region 20q13.2 can be excluded to confer a frequent major gene effect to the expression of IGE spectra with age-related onset from childhood to adolescence. However, in presence of likely genetic heterogeneity, our linkage strategy may not have sufficient sensitivity to detect a susceptibility gene from this region with a relatively weak or rare gene effect. As soon as the sequence variations causing BNFC and the human low-voltage EEG have been identified by positional cloning, further studies will reveal whether these mutations contribute to the phenotypic variance of at least a subgroup of the age-related IGEs with onset beyond the neonatal period. In this case, the possibility of an interactive age-dependent genetic influence on seizure liability from infancy to early childhood should be taken into account.

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